

PRACE POGLĄDOWE

ginekologia

Innovative therapy of ovarian cancer based on overexpression of CD44 receptor

Innowacyjna terapia raka jajnika wykorzystująca nadekspresję receptora CD44

Barbara Bukowska, Agnieszka Marczak

Katedra Termobiologii, Wydział Biologii i Ochrony Środowiska, Uniwersytet Łódzki, Polska

Abstract

Ovarian carcinoma constitutes the main cause of cancer-related death among women. The curability rates remain low despite rapid advances in medicine. Thus, the search for new and improved methods continues, with CD44-targeting as one of them. CD44 is a cell-surface glycoprotein, which binds to its ligand – hyaluronic acid (HA) – and regulates crucial processes such as cell differentiation, proliferation and migration. Overexpression of CD44, observed in many ovarian cancer cells, is used in creating carriers for selective delivery of various drugs (paclitaxel, doxorubicin, camptothecin or cisplatin) to cancer cells.

In this article, we summarized the current state of knowledge regarding CD44-targeting as a new and more efficient way of ovarian cancer treatment, with high potential and promising therapeutic perspectives.

Key words: **targeted therapy / ovarian cancer / CD44 /**

Streszczenie

Nowotwór jajnika stanowi jedną z głównych przyczyn śmierci wśród kobiet. Pomimo gwałtownego rozwoju medycyny jego wyleczalność jest wciąż na bardzo niskim poziomie. Z tego powodu nowe i lepsze metody terapii są wciąż poszukiwane. Jedną z nich jest celowanie w cząsteczkę CD44. CD44 jest glikoproteiną błonową, która wiąże się ze swoim ligandem – kwasem hialuronowym (HA) – i reguluje kluczowe procesy takie jak różnicowanie, proliferacja czy migracja komórek. W wielu komórkach raka jajnika obserwowana jest nadekspresja CD44, co wykorzystuje się przy tworzeniu nośników, aby selektywnie dostarczać leki (paklitaksel, doksorubicynę, kamptotecynę czy cisplatynę) do komórek nowotworowych.

W niniejszym artykule dokonaliśmy podsumowania aktualnego stanu wiedzy dotyczącego terapii celowanej w CD44 jako nowej, skuteczniejszej metody leczenia raka jajnika, cechującej się wysokim potencjałem oraz obiecującymi perspektywami.

Słowa kluczowe: **terapia celowana / CD44 / nowotwór jajnika /**

Adres do korespondencji:

Barbara Bukowska
Katedra Termobiologii, Wydział Biologii i Ochrony Środowiska, Uniwersytet Łódzki
ul. Pomorska 141/143, 90-236 Łódź, Polska
e-mail: barbara.bukowska@onet.pl

Otrzymano: **10.09.2014**
Zaakceptowano do druku: **17.12.2014**

Introduction

CD44 is a cell-surface glycoprotein, which is essential for the proper course of cell proliferation and differentiation. It occurs in both, normal and cancer cells, but overexpression of this receptor is observed in the latter [1]. Notably, CD44 has been high-expressed also in cancer stem cells (CSCs), which constitute a small population of undifferentiated cells with the ability of self-renewal. This small fraction of cancer cells is responsible for relapse, chemoresistance and metastasis [2, 3, 4]. Hyaluronic acid (HA), a linear polysaccharide of alternating D-glucuronic acid and N-acetyl-D-glucosamine units (Figure 1A), which is the only non-sulfated glycosaminoglycan and which occurs primarily *in vivo* as sodium hyaluronate, is the main ligand for CD44 (Figure 1B) [5]. The interaction between those molecules is the base for the regulation of cell proliferation and migration [1]. Therefore, HA-bioconjugates have been proposed as a good way to enhance selective uptake of cytotoxic drugs into cancer cells with the overexpression of the CD44 receptor (Figure 1C) [5]. The dual role of HA has been well-known. This polysaccharide on the surface of the carrier is not only recognized by CD44, but it also provides a hydrophilic coat for the carrier, thereby enhancing its circulation time in the bloodstream [6].

Our article presents the latest data on HA-conjugates applicability in the treatment of ovarian cancer, which is the fifth cause of cancer-related death among women. Despite modern diagnostic and therapeutic approaches, the statistics are still alarming. Early-stage ovarian cancer gives unspecific symptoms which are often confused with gastrointestinal disorders. Thus, the majority of cases are discovered in FIGO stage III or IV, when currently applied therapeutic strategies are mostly ineffective. Standard chemotherapy, the treatment of choice besides surgery, is often insufficient due to the development of multidrug resistance (MDR), even to the most commonly applied drugs like anthracyclines or taxanes [7, 8]. Taking this into account, the search for new methods, which would be efficient especially in cases resistant to other ways of treatment, remains a challenge for contemporary medicine. Owing to CD44 overexpression in ovarian cancer cells, new approaches using this molecule for a targeted therapy have been recently developed [9].

Hyaluronic acid as a 'backbone' for anticancer drugs

The number of antineoplastic drugs delivered to cancer cells in special carriers and directed specifically against the CD44 molecule is substantial. Chief among them is paclitaxel (PTX), a commonly used member of taxanes, whose anticancer efficacy has been documented in various cancer types, including ovarian cancer [10]. Auzenne et al., examined a conjugate (HA-PTX) composed of a hyaluronic acid molecule, which was a backbone for PTX. *In vitro* assays revealed that HA-PTX was cytotoxic against SKOV-3ip and NMP-1 human ovarian carcinoma cell lines, which overexpressed CD44. The efficacy of HA-PTX conjugate was compared to single PTX activity in *in vivo* studies. SKOV-3ip and NMP-1 cells were transplanted into female nude mice and the animals were treated with HA-PTX or free PTX. NMP-1 xenografts turned to be strongly resistant to PTX treatment, while administration of a single-dose of HA-PTX significantly improved survival in mice as compared to controls. Even a single dose of HA-PTX was enough to exhibit antitumor activity

in the SKOV-3ip model. MR (magnetic resonance) studies also showed reduced tumor mass in HA-PTX-treated mice (in both, NMP-1 and SKOV-3ip models), much greater than in control animals. What is also important, HA-PTX conjugates are specifically transported by CD44 receptors, contrary to nonspecific pinocytosis uptake of another PTX conjugate, XYOTAX (PTX linked with poly-L-glutamic acid). Preincubation with free HA stopped the ability of the HA-PTX conjugate to decrease cell survival [11]. Other anticancer drugs, such as doxorubicin or cisplatin, may also be directly attached to HA.

HA as a part of nanoparticles

HA-PTX conjugates are active against different types of cancers, but the PTX/HA ratio is of key importance. High dosages of PTX are believed to decrease the solubility and mask the recognition elements of HA, thus restricting the conjugate activity [12]. The use of additional components might present a solution to this problem. Paclitaxel is often used as a conjugate with a water-soluble N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer. Its efficacy against ovarian carcinoma has been well-established [13]. Journo-Gershfeld et al., observed an enhanced targeting ability to the CD44-overexpressing SKOV-3 ovarian cancer cells when HPMA copolymer was connected with low molecular weight hyaluronan, significantly higher than the toxicity of conjugates of HPMA and PTX, not modified with HA [12].

Similar effects were noted for doxorubicin (DOX). Luo et al., examined two kinds of conjugates: HA-DOX and HA-DOX linked to HPMA copolymer. The latter was much more cytotoxic against SKOV-3 cells than both, the non-targeted HPMA-DOX and the HA-DOX conjugate, but not toward mouse fibroblast NIH-3T3 cells. Those studies confirmed that HA is responsible for selective delivery of anticancer drugs to the CD44 overexpressing cells [14]. Immuno-mediated delivery of doxorubicin was also provided due to liposomes loaded with this anticancer agent, targeted specifically to tumor cells overexpressing the CD44 cell-surface marker. Their activity has been well-established in *in vitro* studies on murine melanoma cell line [15], liver [16], breast [17], and ovarian cancer [18] lines.

Vangara et al., tested yet another CD44-targeting system. They used hyaluronic acid (HA)-decorated poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) nanoparticles, which were loaded with SN-38. This molecule is a derivative of camptothecin and inhibits the activity of topoisomerase I. However, its application is limited due to poor solubility in water and other commonly used solvents. Thus, SN-38 nanomedicine formulations, improving drug delivery to cancer cells, are synthesized, such as PLGA-PEG. Firstly, assays demonstrated that the cellular uptake of PLGA-PEG decorated with HA was much higher in SKOV-3 and OVCAR-8 ovarian cancer cell lines (both exhibit CD44 expression) than in CD44-negative cells. Secondly, carrier coated with HA was significantly more cytotoxic in both tested lines in comparison to non-targeted nanoparticles [19]. Similar conjugates were discovered for targeted delivery of camptothecin [20].

Cisplatin is another chemotherapeutic agent commonly used in ovarian cancer treatment. It also may be a part of conjugates specifically delivering the active substance to ovarian cancer cells overexpressing CD44. Li et al., developed Hyplat microparticles (hyaluronan cross-linking with cisplatin) and tested them

on CD44-positive ovarian cells (OV2008 and A2780). They demonstrated that internalization of Hyplat microparticles is more efficient in cells with high expression of CD44 as compared to controls (UCI101). Also, these authors observed that Hyplat uptake was significantly enhanced in CD44-positive cells, but not CD44-negative cells in comparison to the results obtained for free cisplatin. The conjugate inhibits the growth of A2780 cells inoculated intraperitoneally and improves the survival more effectively than free cisplatin [21]. In turn, Bai et al., tested cisplatin-encapsulating particles coated with maleimide-polyethylene glycol-Poly(D,L-lactic-co-glycolide) (cis-encapsulating-PEG-PLGA) conjugated with CD44 monoclonal antibody. Its efficacy was determined on two CD44-overexpressing ovarian cancer cell lines: CP70 and SKOV-3. It was proven that cis-encapsulating-PEG-PLGA particles targeted to CD44 molecules exhibited higher antiproliferative activity against ovarian cancer cells than free form of cisplatin, cis-encapsulating PLGA particles and cis-encapsulating PEG-PLGA particles without specific antibody [22].

Magnetic nanoparticles are one of the modern classes of carriers and are used for both, diagnostic and therapeutic purposes. Dakdouki et al., demonstrated their efficacy in ovarian cancer. In the first step, *in vitro* studies were undertaken and demonstrated that doxorubicin attached to hyaluronan-coated superparamagnetic iron oxide nanoparticles (HA-SPION) was much more toxic to ovarian cells (both, drug-sensitive and multi-drug-resistant) than free DOX. Additionally, nanoparticles coated with HA provided more rapid uptake by cancer cells in comparison to particles without hyaluronan [23]. Similar effects were observed in *in vivo* studies. Efficacy of DOX-loaded HA-SPIONs was assessed on SKOV-3 ovarian tumor models in nude mice. It was indicated that DOX released from the nanoparticles accumulated in tumor tissue at higher levels than free DOX given intravenously. Also, the distribution of the delivered DOX was wider. Consequently, reduction of tumor growth, delay in tumor development and survival extension in mice were more significant after DOX-HA-SPIONs [24]. The above mentioned findings suggest that HA-SPIONs constitute a promising approach to target CD44-overexpressing ovarian cancer cells.

Also, silica nanoparticles were used to tackle CD44-overexpressing ovarian cancer cells. El-Dakdouki et al., synthesized doxorubicin-loaded, hyaluronan coated silica nanoparticles (SNPs), which contained fluorescent core targeting CD44 molecules. It was observed that the uptake of ligand-free SNPs by the SKOV-3 cells, overexpressing CD44, was much lower than the uptake of hyaluronan coated nanoparticles. Further studies revealed that CD44-mediated endocytosis was the main way of HA-SNPs penetration. Also, nanoparticles with DOX were efficiently delivered to cancer cells [25]. Further studies are necessary to confirm that methods of cancer treatment based on CD44-targeting are more effective.

HA in the fight against MDR

Targeting CD44 molecules may also overcome MDR, which is the major cause of chemotherapy inefficiency. In order to avoid the mediator of MDR, P-glycoprotein (P-gp), phospholipid-based nanoparticle clusters coated with CD44 ligand, glycosaminoglycan hyaluronan, called gagomers (GAGs), were devised. The activity of DOX-loaded GAGs (DOX-GAGs) in ovarian cancer cells overexpressing CD44 was compared with the effective-

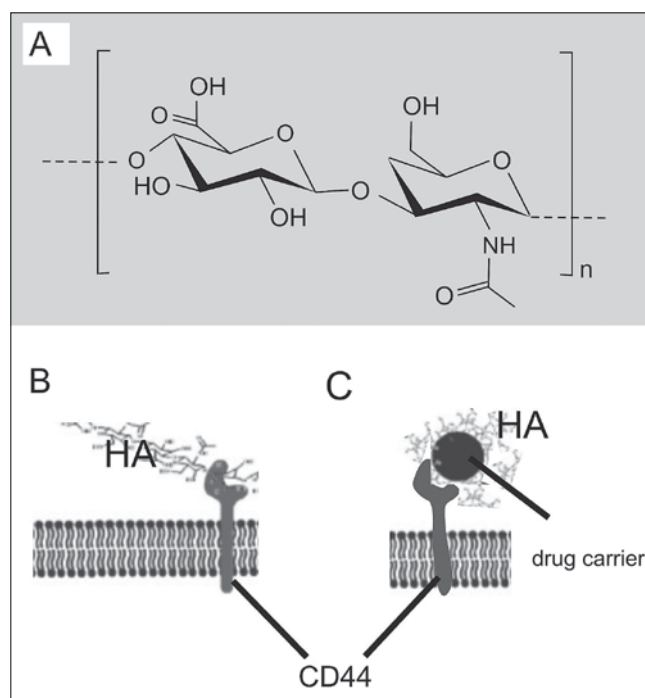


Figure 1. (A) Chemical structure of hyaluronic acid (HA); (B) binding of HA to the receptor CD44, which allow the penetration of HA into cells; (C) use of HA to the transfer of anticancer drug into the cell (Based on [5], modified).

ness of both, liposomal DOX (DOXIL) and drug-free GAGs. It turned out that DOX-GAGs significantly decreased cell viability, whereas DOXIL and free GAGs did not bring therapeutic effects. What is more, further studies based on RNA interference strategy revealed that DOX-GAGs could overcome P-gp-mediated mechanism of MDR. High efficacy of DOX-GAGs in comparison to free DOX was also established in *in vivo* studies in resistant human ovarian adenocarcinoma mouse xenograft model [26].

Conclusions

CD44 molecule offers a wide spectrum of possibilities for improving medical intervention. Different therapeutic strategies using CD44 overexpression might be successfully applied in ovarian cancer treatment. First and foremost, HA can serve as a backbone for anticancer drugs, such as paclitaxel, which are then directed specifically against CD44 molecule on cancer cell surface. Nevertheless, HA may also be a part of more complex carriers consisting of HA, antineoplastic drugs (e.g. paclitaxel, doxorubicin, camptothecin and its derivative SN-38, cisplatin) coated with HPMA or PEG-PLGA. Various researchers confirmed that decorated nanoparticles are more cytotoxic and reveal higher activity against ovarian cancer cells than uncoated ones. The latest methods use superparamagnetic iron oxide and silica nanoparticles loaded with doxorubicin to tackle CD44 overexpressing ovarian cancer cells. Targeting CD44 may also be a way of overcoming MDR. Gagomers, which are phospholipid-based nanoparticle clusters decorated with glycosaminoglycan hyaluronan, have the ability to avoid P-gp and thus may possibly enhance the efficacy of chemotherapy. In our paper, we provided a short summary of using CD44-targeting in ovarian cancer treatment.

Barbara Bukowska, Agnieszka Marczak. *Innovative therapy of ovarian cancer based on overexpression of CD44 receptor.***Oświadczenie autorów:**

1. Barbara Bukowska – autor koncepcji i założeń pracy, przygotowanie manuskryptu i piśmiennictwa – autor zgłaszający i odpowiedzialny za manuskrypt.
2. Agnieszka Marczak – współautor koncepcji i założeń pracy, korekta i akceptacja ostatecznego kształtu manuskryptu.

Źródło finansowania:

Dotacja na finansowanie działalności polegającej na prowadzeniu badań naukowych lub prac rozwojowych oraz zadań z nimi związanych służących rozwojowi młodych naukowców oraz uczestników studiów doktoranckich.

Konflikt interesów:

Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

23. El-Dakdouki MH, Zhu DC, El-Boubbou K, [et al.]. Development of multifunctional hyaluronan-coated nanoparticles for imaging and drug delivery to cancer cells. *Biomacromolecules*. 2012, 13 (4), 1144-1151.
24. El-Dakdouki MH, Xia J, Zhu DC, [et al.]. Assessing the in vivo efficacy of doxorubicin loaded hyaluronan nanoparticles. *ACS Appl Mater Interfaces*. 2014, 6 (1), 697-705.
25. El-Dakdouki MH, Pure E, Huang X. Development of drug loaded nanoparticles for tumor targeting. Part 1: synthesis, characterization, and biological evaluation in 2D cell cultures. *Nanoscale*. 2013, 5 (9), 3895-3903.
26. Cohen K, Emmanuel R, Kisin-Finier E, [et al.]. Modulation of drug resistance in ovarian adenocarcinoma using chemotherapy entrapped in hyaluronan-grafted nanoparticle clusters. *ACS Nano*. 2014, 8 (3), 2183-2195.

References

1. Platt VM, Szoka JC Jr. Anticancer therapeutics: targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor. *Mol Pharm*. 2008, 5 (4), 474-486.
2. Chen J, Wang J, Chen D, [et al.]. Evaluation of characteristics of CD44+CD117+ ovarian cancer stem cells in three dimensional basement membrane extract scaffold versus two dimensional monocultures. *BMC Cell Biol*. 2013, 14, 7. doi: 10.1186/1471-2121-14-7.
3. Chen J, Wang J, Zhang Y, [et al.]. Observation of ovarian cancer stem cell behaviour and investigation of potential mechanisms of drug resistance in three-dimensional cell culture. *J Biosci Bioeng*. 2014, 118 (2), 214-222. doi: 10.1016/j.jbiosc.2014.01.008
4. He QZ, Luo XZ, Wang K, [et al.]. Isolation and characterization of cancer stem cells from high-grade serous ovarian carcinomas. *Cell Physiol Biochem*. 2014, 33 (1), 173-184.
5. Marczak A, Rogalska A. Modyfikacje liposomów – sposób na zwiększenie selektywności chemioterapii. *Problemy Terapii Monitorowanej*. 2010, 21 (2), 107-116.
6. Nehoff F, Parayath NN, Domanovitch L, [et al.]. Nanomedicine for drug targeting: strategies beyond the enhanced permeability and retention effect. *Int J Nanomedicine*. 2014, 9, 2539-2555.
7. Kigawa J. New strategy for overcoming resistance to chemotherapy of ovarian cancer. *Yonago Acta Med*. 2013, 56 (2), 43-50.
8. Maciejczyk A, Surowiak P. Quercetin inhibits proliferation and increases sensitivity of ovarian cancer cells to cisplatin and paclitaxel. *Ginekol Pol*. 2013, 84 (7), 590-595.
9. Zou L, Yi T, Song X, [et al.]. Efficient inhibition of intraperitoneal human ovarian cancer growth by short hairpin RNA targeting CD44. *Neoplasma*. 2014, 61 (3), 274-282.
10. Demain AL, Vaishnav P. Natural products for cancer chemotherapy. *Microb Biotechnol*. 2011, 4 (6), 687-699.
11. Auzenne E, Ghosh SC, Khodadadian M, [et al.]. Hyaluronic acid-paclitaxel: antitumor efficacy against CD44(+) human ovarian carcinoma xenografts. *Neoplasia*. 2007, 9 (6), 479-486.
12. Journo-Gershfeld G, Kapp D, Shamay Y, [et al.]. Hyaluronan oligomers-HPMA copolymer conjugates for targeting paclitaxel to CD44-overexpressing ovarian carcinoma. *Pharm Res*. 2012, 29, 1121-1133.
13. Larson N, Yang J, Ray A, [et al.]. Biodegradable multiblock poly(N-2-hydroxypropyl) methacrylamide gemcitabine and paclitaxel conjugates for ovarian cancer cell combination treatment. *Int J Pharm*. 2013, 454 (1), 435-443.
14. Luo Y, Bernshaw NJ, Lu ZR, [et al.]. Targeted delivery of doxorubicin by HPMA copolymer – hyaluronan bioconjugates. *Pharm Res*. 2002, 19 (4), 396-402.
15. Eliaz RE, Szoka FC Jr. Liposome-encapsulated doxorubicin targeted to CD44: A strategy to kill CD44-overexpressing tumor cells. *Cancer Res*. 2001, 61 (6), 2592-2601.
16. Wang L, Su W, Liu Z, [et al.]. CD44 antibody-targeted liposomal nanoparticles for molecular imaging and therapy of hepatocellular carcinoma. *Biomaterials*. 2012, 33 (20), 5107-5114.
17. Goodarzi N, Ghahremani MH, Dinarvand R. Targeting CD44 by hyaluronic acid-based nano drug delivery systems may eradicate cancer stem cells in human breast cancer. *J Med Hypotheses Ideas*. 2011, 5, 26-31.
18. Vingerhoeds MH, Steerenberg PA, Hendriks JJ, [et al.]. Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma in vitro and in vivo. *Br J Cancer*. 1996, 74 (7), 1023-1029.
19. Vangara KK, Liu JL, Palakurthi S. Hyaluronic acid-decorated PLGA-PEG nanoparticles for targeted delivery of SN-38 to ovarian cancer. *Anticancer Res*. 2013, 33 (6), 2425-2434.
20. Choi KY, Yoon HY, Kim JH, [et al.]. Smart nanocarrier based on PEGylated hyaluronic acid for cancer therapy. *ACS Nano*. 2011, 5 (11), 8591-8599.
21. Li SD, Howell SB. CD44-targeted microparticles for delivery of cisplatin to peritoneal metastases. *Mol Pharm*. 2010, 7 (1), 280-290.
22. Bai MY, Liu SZ. A simple and general method for preparing antibody-PEG-PLGA sub-micron particles using electrospray technique: an in vitro study of targeted delivery of cisplatin to ovarian cancer cells. *Colloids Surf B Biointerfaces*. 2014, 117, 346-353.